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Bile Acids and Lipid Metabolism. I. Stimulation of Bile Lipid Excretion by Various Bile Acids (32855)

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Although considerable information is available on the role of bile acids in the intestinal absorption of lipids, very little is known concerning the function of these compounds in the transport of lipids by other tissues. Rat hepatic bile contains, in addition to bile salts, such lipids as cholesterol, phospholipids and glycerides, as well as bile pigments, and steroids (1). A relationship between bile acids and bile lipids can be inferred from the finding of macromolecular complexes in bile containing these compounds (2). In addition, Kay and Entenman (1), showed a progressive decrease in bile acid and certain bile lipids using bile fistula rats as well as with the isolated, perfused rat liver. With the bile fistula rats, biliary taurocholic acid, phospholipids and free cholesterol decreased whereas the biliary levels of glycerides and esterified cholesterol remained unchanged. With the isolated perfused rat liver the levels of biliary phospholipid and free cholesterol fell to essentially zero 90 min after the start of perfusion. Upon addition of cholic acid to the perfusate, biliary taurocholic acid increased as much as 30-fold, free cholesterol by 2-fold, and phospholipids as much as 50-fold.

In the present paper, data on the initial studies on the role of the bile acids in the synthesis, metabolism, and excretion of bile lipids by the isolated perfused rat liver is reported. It is shown that both primary and secondary bile acids normally present in the enterohepatic circulation of the rat (3) are effective in stimulating biliary lipid excretion by the isolated perfused rat liver.

Methods and Materials. Bile salt preparations. The following bile salts were used: sodium taurocholate (Miles, Ames, Research Division); sodium deoxycholate, special enzyme grade (Mann Research Laboratories); hyodeoxycholic acid (K and K Laboratories); chenodeoxycholic acid, Grade A (Cal-Biochem); sodium lithocholate (Steraloids, Inc.); sodium dehydrocholate (Ames Company); and sodium glycocholate (Mann Research Laboratories). Each preparation used was shown to give a single spot on a thin-layer chromatogram using the techniques of Kritchevsky (4) and Gregg (5). The compounds were also treated by the alkaline hydrolysis technique of Kottke *et al.* (6) and the extracts applied to a thin-layer plate. The free acids again gave only a single spot on the chromatogram.

Solutions for injection into the perfusates were prepared as follows: taurocholate, deoxycholate, and glycocholate were dissolved in isotonic saline; hyodeoxycholate was dissolved in isotonic saline that had been adjusted to pH 8.0 with NaOH; and chenodeoxycholate was dissolved in 20 m*M* sodium bicarbonate. Lithocholate (40 mg) was dissolved in 3 ml of methyl alcohol, combined with 0.25 ml of propylene glycol, and the methanol was evaporated under a stream of nitrogen at 80°C. The propylene glycol–lithocholate solution was mixed with 12 ml of rat plasma that had been cleared of the vasoconstrictor factor (i.e., prepared from blood that had been perfused through a liver for 90 min).

Liver perfusion technique. The apparatus and general techniques used for rat liver perfusions were those described by Brauer *et al.* (7). Livers were taken from ether-anesthetized donor, fed, male Sprague-Dawley rats

TABLE I. Influence of Bile Acids on Biliary Lipid Extraction.[a]

Perfusion series no.	Total perfusion time (min)	Time bile salt infused (min)	Bile salt infused													
			Taurocholate		Glycocholate		Cheno-deoxycholate		Deoxycholate		Hyo-deoxycholate		Lithocholate		Dehydro-cholate	
			FC	PLP	FC	PLP	FC	PLP	FC	PLP	FC	PLP	FC	PLP	FC	PLP
I	60	0	7.4	1.9	2.9	0.7	2.1	2.8	0	3.0	13.1	4.4	4.4	13.0	7.9	1.1
	120	60	13.3	49			53	96	33	31	12.9	69.6	19.0	89	28.8	22.0
	180	120[b]	77	90	49	52	54	115	55	53	44.0	105			35.5	30.4
	240		19.6	56	33	59	70	126	18.4	18.0	33.2	94				
II	60	0	12.6	1.0	3.0	3.9	5.1	9.1	0	3.4	8.9	3.0	3.3	1.0	7.0	0.9
	120	60	11.3	37	6.1	87	111	169	43	53	94	96	10.6	25.0		
	180	120[b]	59	63	134	126	107	203	53	68	92	153	12.8	76	23.7	19.4
	240		49	50		153	220	326	14.3	12.0	90	127			15.8	42.9
III	60	0	4.1	4.9	14.9	4.4	13.6	18.7			5.0	3.7			5.6	0.8
	120	60	153	70	13.3	61	8.5	13.8			91	108			12.9	19
	180	120[b]	175	82	99	100	7.9	64			72	120			27	32
	240		181	87	16.2	87	7.3	56			89	141			58	65

[a] FC = Free cholesterol; PLP = phospholipid phosphorus. Values for FC and PLP are in μg/hour.

[b] Stopped bile salt infusion.

weighing 300–350 gm. Less than 4 min elapsed between tying of the portal vein and re-establishment of circulation in the perfusion system. The perfusion medium was approximately 130 ml of whole rat blood containing about 1 mg of heparin. An initial perfusion period of 60–90 min was used to establish uniform blood and bile flow rates and to eliminate the vasoconstrictor factor present in fresh rat blood (Brauer *et al.* 8). Perfusions were carried out in a chamber maintained at 37°C and a relative humidity of 50–60%. Samples of bile were taken before and after the infusion of bile salt preparations at the time intervals shown in Table I. Bile samples were collected in calibrated tubes. Injection of all bile acid preparations were at a constant rate of 10 mg/hour using a Harvard apparatus double syringe pump and a calibrated syringe. Three replicate experiments were carried out with each bile acid. Blood flow rates ranged from 1.15 to 1.76 ml/min per liver after the initial equilibration period. Bile flow rates were initially about 1 ml/hour. The flow rates were, in some instances, maintained throughout the remainder of the perfusion period. Usually the flow rate fell very gradually during the perfusion, except for a slight increase 30–60 min after beginning the bile salt infusion. A notable exception was found upon infusion of lithocholic acid in which case the bile flow rate showed an early fast decline and during the last interval shown in Table I the rate of flow was less than 0.5 ml/hour.

Analytical methods. Lipids were extracted from the bile samples using 20 volumes of chloroform:methanol (2:1, v/v) per volume of bile at room temperature. The lipids were partitioned with 0.2 volume of 0.05% aqueous calcium chloride solution using the techniques of Folch *et al.* (9). Phospholipid phosphorus in the chloroform phase was determined by the method of Bartlett (10). Free cholesterol was precipitated with digitonin and the amounts were estimated by the ferric chloride method of Zlatkis *et al.* (11). Purity of the bile salt preparations were determined as described above.

Results and Discussion. From the work of Kay and Entenman (1) and Gerber and Remy-Defraigne (12) it was demonstrated that either sodium cholate or sodium taurocholate was effective in increasing the rate of excretion of biliary free cholesterol and phospholipids. The data shown in Table I indicate that other bile acids normally present in the enterohepatic circulation of rats can also stimulate the release of free cholesterol (FC) and phospholipid phosphorus (PLP) into bile of the perfused rat liver. These include two conjugated derivatives of 3α, 7α, 12α-trihydroxycholanic acid (tauro- and glycocholic acids); two dihydroxycholanic acid derivatives, i.e., chenodeoxycholic acid (3α,7α) and deoxycholic acid (3α,12α); and one monohydroxy derivative, lithocholic acid (3α). In addition, two other derivatives of cholanic acid, not normally present in rat blood, namely, triketocholanic acid and hyodeoxycholic acid (3α,6α-dihydroxycholanic acid) were also effective in stimulating bile lipid excretion. Thus, it has been demonstrated that biliary lipid excretion is influenced not only by the predominant rat bile acid, taurocholic, but by other bile acids as well. Conjugation of bile acid with taurine is readily effected by the perfused liver (1). The stimulatory effects of the above bile acids on biliary lipid excretion cannot be ascribed to any specific bile acid structure of the infused acids because the rat liver can rapidly hydroxylate the infused bile acids (except the trihydroxycholanic derivatives) to more polar compounds.

Summary. An increased excretion of biliary free cholesterol and phospholipid was shown to be produced by infusion of various bile acids into the isolated perfused rat liver system. The derivatives studied were the sodium salts of taurocholic, glycocholic, chenodeoxycholic, deoxycholic, lithocholic, hyodeoxycholic, and dehydrocholic acids. Hence, the major bile acids, normally found in the enterohepatic circulation of the rat, can exert stimulatory effects on bile lipid excretion.

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A Vitamin D-Dependent Serum Factor Promoting Calcium Uptake by Bone (32856)

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In 1924, Shipley (1) reported that rachitic rat bone would not exhibit normal calcification when incubated in serum from rachitic rats, but would calcify in normal rat serum. Similar results were obtained with human tissue (2) and fowl tissue (3). However, such experiments were apparently discontinued after Nicolaysen and Jensen (4) reported that in vitamin D-deficient rats, the density of bone calcification was solely related to the amounts of calcium and phosphorus in the blood.

Recent experiments with vitamins D (5,6) and K (7) suggest that these vitamins exert their effects on calcium transport in the intestine and blood coagulation, respectively, by mediating the syntheses of specific proteins. In view of these results, we have repeated Shipley's experiment and have found that normal rat serum contains a vitamin D-dependent factor that stimulates the *in vitro* uptake of calcium by bone.

Materials and Methods. Weanling female rats[1] were kept in the dark, were given distilled water to drink, and were fed *ad libitum* the vitamin D-free, purified diet described by Steenbock and Herting (8). This diet contains normal concentrations of calcium (0.47%, as $CaCO_3$) and phosphorus (0.30%, as NaH_2PO_4) and has a Ca:P ratio of 1.2. The rats were maintained on this diet for 6–8 weeks, even though the concentration of serum calcium was decreased after 3–4 weeks.

There were two different groups of control animals. One control group consisted of normal, female, chow-fed[2] rats having the same body weights as the vitamin D-deficient animals. The second control group consisted of vitamin D-deficient animals which had been fed the purified diet containing vitamin D_2 (0.5 μg (20 IU)/gm of diet) for the last 5 days of the feeding period.

The animals were decapitated, and the blood was collected into 40-ml centrifuge tubes kept at 0°C. The clotted blood was centrifuged at 2°C and sera from similarly treated animals were pooled. Whole femurs, free from adhering tissue, were removed from both deficient and control animals as soon as possible after the death of the animal, and were kept at 0°C on filter paper moistened with saline. The bones were split longitudinally with a scalpel blade prior to incubation.

Calcium in the pooled sera was determined by a fluorometric procedure,[3] inorganic phosphorus was determined by the procedure of Kolb *et al.* (9), and alkaline phosphatase was measured by Phosphatabs.[4] The concentrations of total calcium and inorganic phosphorus in the pooled serum from the deficient animals were adjusted to the concentrations in the normal serum by adding a standard calcium chloride solution (3.0 mg Ca/ml) and

[1] Holtzman Rat Co., Madison, Wisconsin.

[2] Ralston-Purina Co., St. Louis, Missouri.

[3] Manual of Fluorometric Clinical Procedures, G. K. Turner Associates, Inc., 2524 Pulgas Avenue, Palo Alto, California.

[4] Warner-Chilcott, Morris Plains, New Jersey.